# INVESTIGATING THE RELATIONSHIP BETWEEN MANAGEMENT PRACTICES AND GENETIC DIVERSITY: A POPULATION GENETICS STUDY OF PEN SHELL CLAM (*Atrina tuberculosa*) IN THE GULF OF CALIFORNIA, MEXICO

by

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**Abstract**

*The management of commercial fisheries is complicated by the movement of the resource of interest among areas with different management practices. When a species is heavily harvested in one portion of its range, the effects are translated to other fisheries in terms of a decline in abundance, or in some cases, a loss of genetic diversity. Conserving genetic diversity is important, especially in commercially harvested populations, because it provides the population with greater resilience to changing environmental conditions. One popular strategy that fisheries managers use to alleviate the differences in fishing pressures across the entire range of a species is the establishment of marine protected areas. MPAs also act to conserve biodiversity within their boundaries, and many areas of the world's highest marine biodiversity occur in developing countries where commercial fishing is practiced at small scales. Understanding the interplay between MPAs and small-scale fisheries will allow for the most comprehensive management decisions. My research uses molecular techniques to investigate the levels of genetic diversity between two commercially harvested bivalve populations in the Gulf of California, Mexico in order to evaluate the effectiveness of a community-based MPA in terms of a potential genetic benefit to an open-access fishery. The results show no significant genetic structure or difference in levels of genetic diversity between the two populations, and the lack of private alleles indicates that there is positive gene flow between these two areas by means of larval transport. Despite the influx of larvae from the MPA to the open-access fishery, pen shell abundances continue to decline, and these data suggest that the presence of an MPA adjacent to this open-access fishery is not enough to sustain the population under current harvesting practices.*

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## Introduction

The management of commercial fisheries is complicated by the fact that harvested species often move between areas with different management practices. A species may migrate between feeding grounds and spawning grounds, like tuna or marlin; or the larvae and juveniles may travel long distances driven via ocean currents, as is the case with many bivalve species. When a motile species is heavily harvested in one portion of its range, the effects are translated to other locations as a decline in abundance. This overexploitation occurs in many fisheries due to a lack of either access control mechanisms or enforcement of harvesting limitations (Defeo and Castilla 2012), and many coastal communities now recognize the need for an increase in protection of locally important commercial marine resources.

One popular solution to this management complexity is the establishment of marine protected areas (MPAs) where harvesting is either prohibited or highly restricted. These MPAs can take the form of individual sites or networks of protected areas based on species dispersal range that act as "stepping stones" between fisheries (Harrison et al. 2012, Planes et al. 2009). In many cases, the presence of marine reserves near commercially important fisheries can be beneficial because it allows for the export of larvae or juveniles from the reserve into the fishery areas (Harrison et al. 2012, Planes et al. 2009, Underwood et al. 2013).

Support for the establishment of MPAs has grown as studies have shown that these areas also act to conserve biodiversity within the boundaries of the reserve, as well as to promote fishery management objectives (Roberts et al. 2005). A critical

area for the development of MPAs is areas of high marine biodiversity, which often occur in developing countries (Marinesque et al. 2011), and the harvesting of marine resources in developing countries is frequently dominated by small-scale fisheries (SSFs). Further, SSFs employ approximately 98% of the world's fishers and these fishers bring in nearly half of the world's annual marine catch (Berkes et al. 2001). Understanding the interplay between MPAs and SSFs will allow for more comprehensive management decisions regarding these commercially important fisheries.

Here, we focus on a case study of fisheries in the northern Gulf of California, an area of high marine biodiversity due to its geologic history (Lluch-Cota et al. 2007) and characterized by commercial dominance of small-scale fisheries (Cinti et al. 2010). Management of these fisheries varies greatly from community to community. For example, two communities just off the western coast of the state of Sonora both harvest the commercially important pen shell clam (*Atrina tuberculosa*), with one community operating under an open-access-by-permit scheme, while the neighboring fishery practices access controls to outsiders by marine tenure rights granted to the local community (Basurto et al. 2012). In this case study, the fishery with controlled access can be considered to act as a community-based MPA; fishing occurs in the area, but the regulations on access and harvesting limitations are such that they are in line with the unofficial definition of MPA used by the Center for Biological Diversity, which defines an MPA as an area "which has been reserved by legislation or other effective means, including custom, with the effect that its marine and/or coastal biodiversity enjoys a higher level of

protection than its surroundings" (fao.org). While these two areas are directly adjacent, the open access fishery has been reporting a decline in abundance and smaller harvests of pen shell over the last 10 years (Basurto et al. 2012). My research investigates the genetic diversity at sites in these two fisheries to see if there is evidence of gene flow between these sites. These data can then be used to evaluate the effectiveness of an adjacent MPA in terms of a positive benefit to the open-access fishery.

I. Study species and area

The species under consideration in this study is the pen shell, *Atrina tuberculosa*, locally known as "callo de riñón" (Yee 2013). It is a sessile, benthic bivalve mollusk, which is harvested regularly by divers for the adductor muscle, a small part of the entire visceral mass of the animal. Pen shells reproduce by year-round broadcast spawning with two peaks, in June and November (Basurto, personal communication). Sexual maturity is reached when the animal is at about 10 cm in shell length, and because the great majority of individuals harvested exceed 20 cm in shell length, it can be assumed that most harvested individuals had the chance to reproduce at least once (Basurto 2008). The ciliated larvae, known as trochophores, can remain in the water column for a period of weeks (Campbell et al. 2008), suggesting the ability of transport between locations. However, adult pen shells bury themselves in the sand, and fishers must dive to the bottom and use a hook to extract them. Another species of pen shell is present in the study area and also harvested year-round by fishermen, *Pinna rugosa*, locally known as "callo redondo,"

however this species commands a much lower monetary value than *A. tuberculosa* (10 pesos/kilo and 90 pesos/kilo respectively, or $0.77 and $6.94 US dollars) (Yee 2013). Fishers in both communities can identify both species visually before harvesting and will preferentially harvest *A. tuberculosa* until abundance is low and then switch temporarily to the harvest of *P. rugosa*.

The study area is located in the western part of the Gulf of California, near the area of Tiburón Island (Figure 1). The Seri community occupies the two towns of El Desemboque and Punta Chueca, the latter of which lies 30 km to the north of Kino village. The Seri harvest pen shells in the 41km long Infiernillo Channel, which is shallow (average depth 5.5m) and has extensive eelgrass (*Zostera marina*) beds (Basurto and Coleman 2010). A semi-diurnal "tidal pulse" brings water into and out of both the northern and southern openings of the channel twice per day (Basurto, personal communication). The Kino pen shell fishery is located in Bahía de Kino, and based out of Kino village. Kino is the closest coastal town to the city of Hermosillo, the capital of Sonora, and is an important port for many major

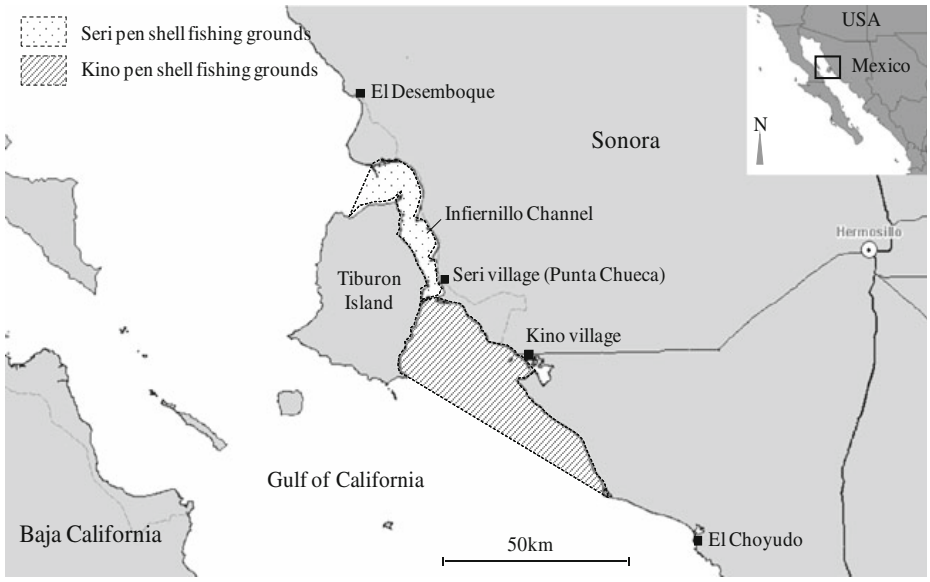



**Figure 1. Map of the study area showing marine territory controlled by the Seri and Kino peoples (from Basurto et al. 2012)**

national and international seafood markets (Basurto et al. 2012). In contrast to the Infiernillo Channel, Bahía de Kino is a deeper, open bay. There are no eelgrass beds in the bay because of the depth, and the bay is also subject to mixed semi-diurnal tides.

II. Human behavioral component

These two fishing communities differ greatly in terms of their historical origin as well as their sense of identity. The Seri community is made up of an indigenous Mexican tribe, the Comcaác people, who have occupied the Sonoran desert and Tiburón Island for thousands of years (Basurto and Coleman 2010). Traditionally, they were a nomadic group of seafaring hunter-gatherers before settling in the early 20th century in the two villages of El Desamboque and Punta Chueca (Basurto 2008), as well as establishing permanent residence on Tiburón Island. The current population stands at about 600 individuals. The Seri have a long history of conflict with European settlers and *mestizo*[1] Mexicans, who have repeatedly tried to remove the Seri with the intent of controlling the land and gaining access to the marine resources in the Infiernillo Channel (Basurto et al. 2012). In contrast, most of the inhabitants of Kino village are recent (~1920s) immigrants to the area. They number about 5,000 individuals (Cinti et al. 2010), and many are of mixed European and native decent. This lack of cultural identity means that most Kino fishermen feel neither the sense of community heritage nor the protectiveness over territory that the Seri do (Basurto 2008).

[1] A person of combined European and Native American descent

These two communities do share similarities, however, in the ways that some members of the community make their livings. Both regions emerged as permanent fishing communities in the 1920s and rapidly depleted stocks of commercially valuable species, including sea turtles, sharks, and large fin fish, and were then forced to begin harvesting species of lower trophic levels that were once considered "trash," such as triggerfish, jellyfish, and sea cucumber, around the early-1970s (Basurto et al. 2012). At that time pen shells were found to be in great abundance in both fishing areas; and by 1978, pen shells came to be so valuable that fish buyers would often cover the costs of fishing supplies for the divers (Basurto 2006). Pen shells are harvested by divers using rudimentary, home-made diving equipment, called hookahs (Figure 2), which are made of an air compressor, the engine of a commercial paint sprayer, and a beer keg as the air reserve tank (Basurto 2006). Divers will typically spend around 4 hours per day in the water, trying to harvest 30-35 kilos (Yee 2013).

**Figure 2. Hookah diving equipment (from Basurto 2006).**

## III. Institutional framework

The most marked difference between these two fisheries is in the legislative governance and the rules-in-use controlling the harvest of pen shells; in this respect, these two fisheries are almost complete opposites, and it is this difference that is thought to be the main driver in the discrepancy in pen shell abundance between the two sites.

However, until 1975 both communities were unsustainably fishing and driving commercially important stocks down. The Seri were fishing under the permit scheme defined by the first Mexican fisheries law, established in 1925 (Basurto et al. 2012), and the entire Seri community fished under one permit as a fishing cooperative. In 1975, the Seri were granted exclusive property rights over Tiburón Island and its adjacent waters by the National Institute for Indigenous Affairs in an effort to preserve the cultural identity of some of the smallest of Mexico's native groups (Basurto et al. 2012). The remoteness of the Seri community limited the influence of the Mexican federal government and forced the Seri to organize a local government to control access to and restrict use of their marine resources.

As it became apparent that the fishing in the Infiernillo Channel was much more productive, pressure began to mount for the Seri to allow outsider use of their fishing grounds. The Seri decided that it would be economically practical to allow a small number of outside fishers to come in, and between 1984 and 1987, they developed a set of rules-in-use to govern the fishing activities of outsiders (Basurto et al. 2012). First off, outside fishers must make direct monetary payment to the

acting Seri government officials. This fee is retained by the Seri governor and his family as salary for the position because no formal taxes or federal funding is present. Second, outside fishers must hire at least one Seri as a member of their fishing crew and this crew member is to be paid an equal share of the catch as compared to other members of the crew. This ensures that a portion of the profit made by the outside boat will remain within the Seri community and that the Seri crew members are not taken advantage of. Finally, no fishing (by either outside boats or Seri boats) is to take place in "culturally sensitive" areas. These areas are shallow sandbars close to the shore, and their use is restricted to elderly members of the Seri tribe and children as a way of perpetuating traditional Seri cultural practices. These rules-in-use, as well as enforcement by an informal group of Seri members known as the *Guardia Tradicional* (Seri Traditional Guard), have allowed for a stable annual harvest of pen shells for the past 30 years (Basurto et al. 2012).

In Kino village, however, harvesting is still regulated under the permit scheme defined by the fisheries law enacted in 1925. Permits are issued by the National Commission of Fisheries and Aquaculture (CONAPESCA), which is the primary agency in charge of regulation and enforcement at the national level (Basurto et al. 2012). At the state level, the Ministry of Agriculture, Livestock, Fisheries, Food and Rural Development (SAGARPA) coordinates the administration and management of these regulations (Basurto et al. 2012). Under this system, in order to obtain a permit for fishing (any species), one must have a boat to register, but there is no limit on the number of permits an individual may hold (Basurto et al. 2012). This requirement for boat ownership and permitting process prevents many

young fishermen from entering into the market as they have neither the resources to obtain a boat, nor the education to deal with the bureaucratic process of applying for a permit (Cinti et al. 2010). Instead, these regulations concentrate the fishing permits in the hands of wealthier community members, mainly the fish-buyers, who hire fishermen to work under their permits. The fisheries law also states that in order to sell catch commercially, one must have a permit. So, the fishermen that are hired workers on the boats of the fish buyers sell their catch "under the table" to the owners of the boat. This exploitative cycle comes full-circle because the fish-buyers/permit holders/wealthy community members are generally the ones who own the local markets where the fishermen must buy not only necessary household items but also the equipment needed for fishing (i.e. wetsuits, dive masks, etc.). Under this system, most of the money becomes concentrated in the hands of the fish-buyers, intensifying social inequities (Cinti et al 2010). Further, there is no incentive to reduce fishing pressure because when pen shell abundance drops in Kino Bay, the fishers either switch temporarily to another species or attempt to gain access to the Infiernillo Channel.

In this study, I use molecular techniques to investigate the levels of genetic diversity between these two neighboring fisheries. By measuring the lengths of polymorphic microsatellite regions, I will be able to assess differences in allelic diversity as well as population structure for pen shells in this environment; and these data can then be used in the context of evaluating this community-based MPA's efficacy in sustaining an adjacent fishery. I hypothesize that we will find

population connectivity, given that the two fisheries are in such close geographic proximity; however, I also would anticipate depressed levels of genetic diversity in the overexploited Kino population.

## Methods

I. Tissue Samples

Samples of *Atrina tuberculosa* were collected between October 2011 and March 2012 at seven sites in the Gulf of California. Six of the sites were located within the Infiernillo Channel with the number of individuals given in parentheses: (Punta Ona (n = 64), Media Luna (n = 30), Paredones (n = 77), Xpatacl (n = 25), Punta Chueca (n = 40), and Coospoj (n = 106)). One site (Kino (n = 59)) was located in Kino Bay, 30 km to the south of the Infiernillo Channel (Figure 3). Pen shells were harvested by local, commercial fishermen, diving with a hookah apparatus and detaching the pen shells from the substrate using a fishing hook. Samples were obtained from locations that are frequently visited by local fishermen. The number of samples collected at each site was scaled by the abundance of pen shell at each location, with a minimum of 25 individuals per site. Intact pen shells were preserved on ice during transport to the

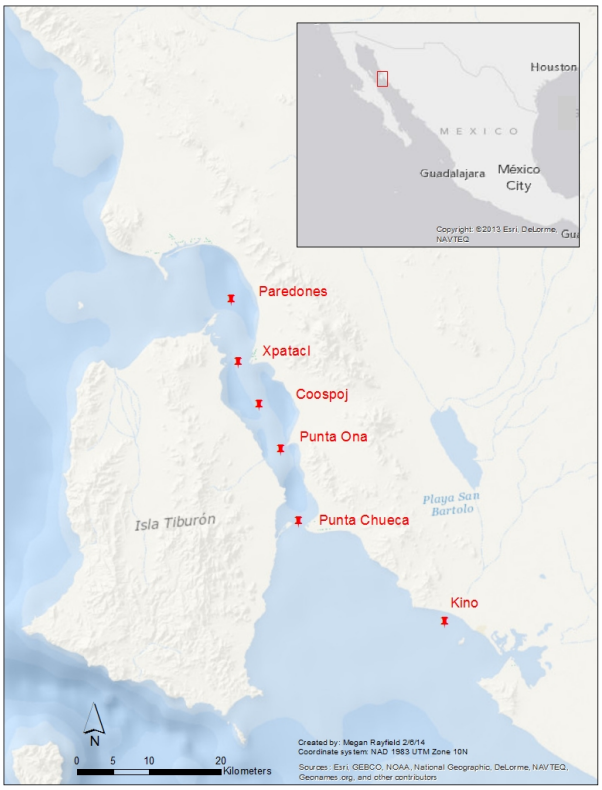



**Figure 3. Map of the study area**

Duke Marine Lab (Beaufort, NC). Adductor muscle tissue samples were then dissected, preserved in ethanol, and stored at room temperature.

II. DNA Extraction, PCR Amplification, and Electrophoresis

Total genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega 2010) for the Kino and Coospoj populations and a CTAB DNA extraction method for the remaining populations. Sixteen primer sets, developed at the Duke Marine Lab (Schultz et al., in review) were used to screen all samples. Thirteen primer sets produced discernible peaks for greater than 70% of individuals within six populations (the Media Luna population was discarded because of a lack of sufficient PCR product) and were used for further analysis. Primer details can be found in Table 1.

PCR was performed in 20 µL reactions containing 2 µl DNA, 2 µl 10x PCR Buffer (200 mM Tris, pH 8.8; 500 mM KCl; 0.1% Triton X-100, 0.2 mg/ml BSA), 1.6 µl 25 mM $MgCl_2$, 1.6 µl 2.5 mM dNTPs, 0.2 µl 10 µM Forward primer, 0.8 µl 10 µM Reverse primer, 0.8 µl 10 µM labeled (FAM, NED, PET, or VIC) T3 primer (Eurofins; Applied Biosystems), and 0.2 µl Taq DNA polymerase. PCR products were indirectly labeled using Forward primers with 5'-T3 tags (ATTAACCCTCACTAAAGGGA; not shown in Table 1) and fluorescently labeled T3 primers. Reactions were thermocycled under the following conditions: 94 °C 4 min, 25 cycles of 94°C 15s, 62°C 15s, 72°C 30s, 8 cycles of 94°C 15 s, 53°C 15s, 72°C 30s; final extension at 72°C for 5 min. PCR products (2 µl) were mixed with 0.05 µl DNA Orange (MCLab), 0.05 µl 10 mg/ml salmon sperm DNA, and 8.95 µl water. The samples were denatured at

95°C for 10 minutes and chilled on ice. Size-fragment analysis was conducted on an ABI 3730xl DNA Analyzer (Applied Biosystems).

Table 1. Characteristics of primer sets used for analysis

| Locus | Repeat motif | Primer Sequence (5'-3') | # alleles | Size range (bp) |
|---|---|---|---|---|
| *At1* | $(ATGC)_5$ | F: TCAAATTTTCCCCGATTATCA | 10 | 178-267 |
| | | R: TCCGGACTTTAGGACCTTCA | | |
| *At4* | $(ATTT)_4$ | F: AGCAGCAGCAACAACAACAG | 1 | 198 |
| | | R: CTGAGTGTCGGACATTTGGT | | |
| *At5* | $(AAAT)_7$ | F: CAGAGAATCCGCCCATAAAA | 36 | 219-265 |
| | | R: ACACTTAGCCGGCTTTCGTA | | |
| *At7* | $(ACAG)_4$ | F: CAACCCTTGCAAGTTTACCTC | 3 | 252-260 |
| | | R: TGCTTGATAGAGAGCAAAATGA | | |
| *At8* | $(ATCT)_{11}$ | F: GCCGTTGAACAGACTCACTG | 28 | 186-260 |
| | | R: CGGACGGACAGACAGACAG | | |
| *At12* | $(AAAC)_4$ | F: TTGGCGGGTTACTAAGCAAG | 9 | 230-291 |
| | | R: CAGTTGACTGAATCGCGAAA | | |
| *At13* | $(ACAT)_4$ | F: GGGTGTCACAAGACAATTTGC | 5 | 219-229 |
| | | R: TGGGACGTTAAACAACATTGC | | |
| *At17* | $(ATCT)_5$ | F: TGGCCTGAGAGATGGCTATT | 1 | 169-256 |
| | | R: TTCTACAGTGAAATTAAGGTGCAT | | |
| *At18* | $(ATCT)_4$ | F: ATACAAACCAAACGCCGAAA | 2 | 172-176 |
| | | R: CGATGCACGACAGACAAAAC | | |
| *At27* | $(CTGT)_8$ | F: TGGCTAGCTGTCCATCTGTCT | 5 | 87-132 |
| | | R: GATTGATGGCCTGATGGATT | | |
| *At31* | $(AAAT)_7$ | F: AAGGTTGGGCACATGAACAG | 13 | 187-243 |
| | | R: TCCATATTAAACGCCGTGTG | | |
| *At36* | $(AATT)_4$ | F: TGCAGACCTAGGACGTGATG | 7 | 194-215 |
| | | R: GGGCAAAATTTACCTCCAGA | | |
| *At46* | $(AGAT)_7$ | F: CCTCCCAGCAAACACATACC | 13 | 189-241 |
| | | R: TCCTCCGGTCATCCATCTAC | | |

III. Fragment Analysis and Statistics

Chromatograms were scored using Genemarker v1.8 (SoftGenetics). The data set was considered complete when data had been recorded for more than 70% of individuals in each population at each marker. The data were arrayed in a spreadsheet, and the program CONVERT (Glaubitz 2004) was used to create input data files for the different statistical software programs. The data were tested for deviations from Hardy-Weinberg equilibrium using GenAlEx 6.5 (Microsoft Corporation, Richmond, VA) and were also examined for the presence of null alleles, possible scoring error due to stuttering, and large allele dropout using Micro-Checker 2.2.3 software (Van Oosterhout 2004).

Various genetic diversity parameters, such as total alleles (A), observed heterozygosity ($H_O$), and expected heterozygosity ($H_E$) were calculated using GenAlEx 6.5 (Peakall and Smouse 2014). Allelic richness ($R_S$) and inbreeding coefficient ($F_{IS}$) were calculated using FSTAT (Goudet 1995), with tablewide levels of significance set to 5%. Analysis of molecular variance (1000 permutations), pairwise $F_{ST}$ (100 permutations, 0.05 significance level), and exact test of population differentiation (100,000 steps in Markov chain, 10,000 dememorization steps, 0.05 significance level) were calculated using Arlequin 3.5.1.3 (Excoffier and Lischer 2010).

A pairwise genetic distance matrix was calculated using GenAlEx 6.5, with missing data interpolated, and this matrix was used as the input for a principal components analysis with data standardization. A Bayesian approach to investigate population structure was performed using the program STRUCTURE 2.3.4

(Pritchard et al. 2000), in which five tests were run (K=2, K=3, K=4, K=5, K=6, where K is the number of assumed populations into which the algorithm bins individuals) with a burn-in period length of 5,000, the number of Markov chain Monte Carlo repetitions set to 5,000, and assuming an admixture model.

**Results**

I. Genetic diversity within populations

A very large proportion of the loci tested, except At5, were found to deviate from Hardy-Weinberg equilibrium. Microchecker results indicate the probable presence of null alleles at all loci except At13, At18, and At27.

Across 11 out of 13 loci, the Paredones population (the northernmost population and farthest from the Kino site) contained either the highest or second highest number of total alleles, but this population displayed relatively high allelic richness at only 3 loci (Table 2).

The Kino population displays relatively high allelic diversity across all loci and contains private alleles at markers At18, At36, and At46 (Figure 4). Private alleles were also found in Paredones (At1, At5, At7, and At8), Xpatacl (At17), and Punta Ona (At1, At4, At7, At27, At46).

Marker At8 was the most polymorphic locus, containing the highest number of alleles across all populations, and only one allele at this marker is private to a single population (Figure 5). In contrast, marker At18 was the least polymorphic locus, containing only three alleles, one of which is private to the Kino population.

Table 2. Summary of genetic diversity statistics of *A. tuberculosa* populations, showing total alleles (A), allelic richness ($R_S$), observed heterozygosity ($H_O$), expected heterozygosity ($H_E$), and inbreeding coefficient ($F_{IS}$).

| | Punta Ona | Paredones | Kino | Xpatacl | Punta Chueca | Coospoj | Total |
|---|---|---|---|---|---|---|---|
| *n* | 64 | 77 | 51 | 25 | 40 | 60 | 317 |
| <u>At_01</u> | | | | | | | |
| Allele range | 255-271 | 252-270 | 254-270 | 254-265 | 252-270 | 254-265 | 252-271 |
| *A* | 10 | 13 | 10 | 5 | 9 | 8 | 14 |
| $R_S$ | 7.647 | 8.082 | 7.860 | 4.609 | 7.255 | 7.037 | 8.055 |
| $H_O$ | **0.214*** ** | **0.242*** ** | **0.357*** ** | 0.333 | **0.400*** ** | **0.292*** ** | 0.306 |
| $H_E$ | 0.740 | 0.757 | 0.754 | 0.333 | 0.712 | 0.584 | 0.647 |
| $F_{IS}$ | 0.715 | 0.685 | 0.535 | 0.024 | 0.450 | 0.545 | 0.492 |
| <u>At_04</u> | | | | | | | |
| Allele range | 183-201 | 198-200 | 198-200 | 198-200 | 198-200 | 183-201 | 183-201 |
| *A* | 6 | 3 | 2 | 3 | 3 | 5 | 6 |
| $R_S$ | 3.891 | 2.712 | 1.957 | 2.990 | 2.815 | 3.258 | 3.106 |
| $H_O$ | **0.145*** ** | **0.065*** ** | **0.000*** ** | **0.167*** ** | **0.075*** ** | **0.086*** ** | 0.090 |
| $H_E$ | 0.333 | 0.271 | 0.130 | 0.292 | 0.363 | 0.193 | 0.264 |
| $F_{IS}$ | 0.570 | 0.763 | 1.000 | 0.446 | 0.798 | 0.561 | 0.690 |
| <u>At_5</u> | | | | | | | |
| Allele range | 220-258 | 220-263 | 220-263 | 230-255 | 226-263 | 230-255 | 220-263 |
| *A* | 17 | 22 | 19 | 12 | 18 | 15 | 25 |
| $R_S$ | 12.836 | 14.339 | 12.882 | 11.719 | 13.440 | 11.013 | 13.584 |
| $H_O$ | 0.808 | **0.892*** | 0.878 | 0.667 | 0.711 | 0.736 | 0.782 |
| $H_E$ | 0.901 | 0.911 | 0.886 | 0.846 | 0.904 | 0.867 | 0.886 |
| $F_{IS}$ | 0.113 | 0.029 | 0.021 | 0.239 | 0.226 | 0.131 | 0.759 |
| <u>At_7</u> | | | | | | | |
| Allele range | 254-262 | 248-262 | 254-262 | 258-262 | 254-262 | 254-262 | 248-262 |
| *A* | 5 | 5 | 3 | 2 | 3 | 3 | 6 |
| $R_S$ | 4.092 | 3.590 | 2.089 | 2.000 | 2.322 | 2.278 | 3.402 |
| $H_O$ | **0.235*** ** | **0.121*** ** | 0.083 | 0.278 | 0.125 | **0.154*** ** | 0.166 |
| $H_E$ | 0.432 | 0.528 | 0.081 | 0.239 | 0.118 | 0.177 | 0.263 |
| $F_{IS}$ | 0.463 | 0.435 | 0.265 | 0.456 | 0.342 | 0.314 | 0.381 |
| <u>At_8</u> | | | | | | | |
| Allele range | 188-254 | 186-258 | 186-254 | 188-254 | 186-258 | 186-258 | 186-258 |
| *A* | 24 | 26 | 24 | 18 | 16 | 25 | 30 |
| $R_S$ | 15.640 | 16.265 | 16.632 | 15.811 | 12.687 | 16.604 | 17.016 |
| $H_O$ | **0.750*** ** | **0.882*** ** | **0.920** ** | **0.826*** | **0.775** ** | **0.830*** | 0.831 |
| $H_E$ | 0.918 | 0.925 | 0.926 | 0.909 | 0.889 | 0.935 | 0.917 |
| $F_{IS}$ | 0.192 | 0.054 | 0.017 | 0.113 | 0.141 | 0.128 | 0.108 |
| <u>At_12</u> | | | | | | | |
| Allele range | 231-245 | 229-241 | 229-245 | 229-241 | 229-241 | 229-245 | 229-245 |
| *A* | 6 | 6 | 7 | 5 | 5 | 7 | 7 |
| $R_S$ | 4.701 | 4.605 | 5.470 | 4.922 | 4.693 | 5.079 | 5.020 |
| $H_O$ | **0.358*** ** | **0.317*** ** | 0.467 | **0.348** ** | **0.378*** ** | **0.431*** ** | 0.383 |
| $H_E$ | 0.658 | 0.555 | 0.626 | 0.619 | 0.565 | 0.638 | 0.610 |
| $F_{IS}$ | 0.463 | 0.435 | 0.265 | 0.456 | 0.342 | 0.314 | 0.379 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| At_13 | | | | | | | |
| Allele range | 218-228 | 218-228 | 197-227 | 218-226 | 218-226 | 197-226 | 197-228 |
| *A* | 6 | 6 | 4 | 3 | 4 | 5 | 8 |
| $R_S$ | 4.255 | 3.852 | 2.895 | 2.943 | 3.447 | 4.239 | 4.089 |
| $H_O$ | 0.327 | **0.121*** ** | **0.102*** ** | 0.227 | **0.184*** | **0.208*** | 0.195 |
| $H_E$ | 0.306 | 0.251 | 0.205 | 0.208 | 0.218 | 0.297 | 0.247 |
| $F_{IS}$ | -0.059 | 0.526 | 0.509 | -0.071 | 0.167 | 0.282 | 0.226 |
| At_17 | | | | | | | |
| Allele range | 185-187 | 185-187 | 185-198 | 173-185 | 185 | 185-198 | 173-198 |
| *A* | 2 | 2 | 3 | 2 | 1 | 3 | 4 |
| $R_S$ | 1.651 | 1.575 | 2.394 | 2.000 | 1.000 | 3.548 | 2.469 |
| $H_O$ | **0.017*** ** | **0.014*** ** | **0.025*** ** | 0.056 | 0.000 | **0.071*** ** | 0.031 |
| $H_E$ | 0.050 | 0.043 | 0.162 | 0.054 | 0.000 | 0.236 | 0.091 |
| $F_{IS}$ | 0.663 | 0.663 | 0.849 | 0.000 | NA | 0.600 | 0.555 |
| At_18 | | | | | | | |
| Allele range | 174-178 | 174-178 | 166-178 | 174 | 174-178 | 174-178 | 166-178 |
| *A* | 2 | 2 | 3 | 1 | 2 | 2 | 3 |
| $R_S$ | 1.785 | 1.481 | 2.089 | 1.000 | 1.837 | 1.687 | 1.684 |
| $H_O$ | **0.037*** ** | 0.033 | 0.083 | 0.000 | 0.079 | 0.065 | 0.050 |
| $H_E$ | 0.071 | 0.032 | 0.081 | 0.000 | 0.076 | 0.063 | 0.054 |
| $F_{IS}$ | 0.488 | -0.008 | -0.025 | NA | -0.028 | -0.025 | 0.080 |
| At_27 | | | | | | | |
| Allele range | 86-132 | 86-132 | 86-132 | 86-132 | 86-132 | 86-132 | 86-132 |
| *A* | 7 | 4 | 3 | 4 | 5 | 3 | 7 |
| $R_S$ | 3.884 | 3.466 | 2.405 | 3.777 | 4.314 | 2.766 | 3.398 |
| $H_O$ | **0.448*** ** | **0.478*** ** | 0.429 | **0.857*** ** | **0.289*** ** | 0.608 | 0.518 |
| $H_E$ | 0.549 | 0.567 | 0.510 | 0.552 | 0.588 | 0.519 | 0.547 |
| $F_{IS}$ | 0.192 | 0.165 | 0.171 | -0.535 | 0.517 | -0.041 | 0.078 |
| At_31 | | | | | | | |
| Allele range | 186-242 | 186-242 | 186-239 | 186-238 | 186-242 | 186-218 | 186-242 |
| *A* | 16 | 20 | 19 | 10 | 17 | 8 | 27 |
| $R_S$ | 10.497 | 11.338 | 11.868 | 9.659 | 11.690 | 7.114 | 11.457 |
| $H_O$ | **0.577*** ** | **0.694*** ** | **0.761*** ** | 0.737 | 0.658 | **0.444*** ** | 0.645 |
| $H_E$ | 0.801 | 0.847 | 0.840 | 0.831 | 0.828 | 0.729 | 0.813 |
| $F_{IS}$ | 0.289 | 0.189 | 0.105 | 0.140 | 0.218 | 0.400 | 0.224 |
| At_36 | | | | | | | |
| Allele range | 205-215 | 205-218 | 197-218 | 205-215 | 205-215 | 207-215 | 197-218 |
| *A* | 9 | 8 | 7 | 6 | 6 | 7 | 11 |
| $R_S$ | 6.706 | 5.307 | 5.282 | 5.011 | 5.271 | 6.102 | 6.397 |
| $H_O$ | **0.152*** ** | **0.183*** ** | **0.093*** ** | **0.240** ** | **0.100*** ** | **0.130*** ** | 0.150 |
| $H_E$ | 0.718 | 0.723 | 0.640 | 0.473 | 0.614 | 0.607 | 0.629 |
| $F_{IS}$ | 0.792 | 0.750 | 0.858 | 0.508 | 0.841 | 0.674 | 0.732 |
| At_46 | | | | | | | |
| Allele range | 187-243 | 187-239 | 184-227 | 187-231 | 186-231 | 187-239 | 184-243 |
| A | 17 | 16 | 13 | 12 | 13 | 14 | 21 |
| $R_S$ | 11.747 | 10.747 | 10.055 | 10.755 | 10.655 | 10.741 | 11.442 |
| $H_O$ | **0.644*** ** | **0.629*** ** | **0.721*** ** | **0.583*** | **0.710** ** | 0.648 | 0.656 |
| $H_E$ | 0.828 | 0.849 | 0.816 | 0.813 | 0.807 | 0.805 | 0.820 |
| $F_{IS}$ | 0.230 | 0.266 | 0.128 | 0.302 | 0.137 | 0.276 | 0.223 |

Departure from Hardy-Weinberg expectations is indicated by bold type (*P<0.05, **P<0.01, ***P<0.001).

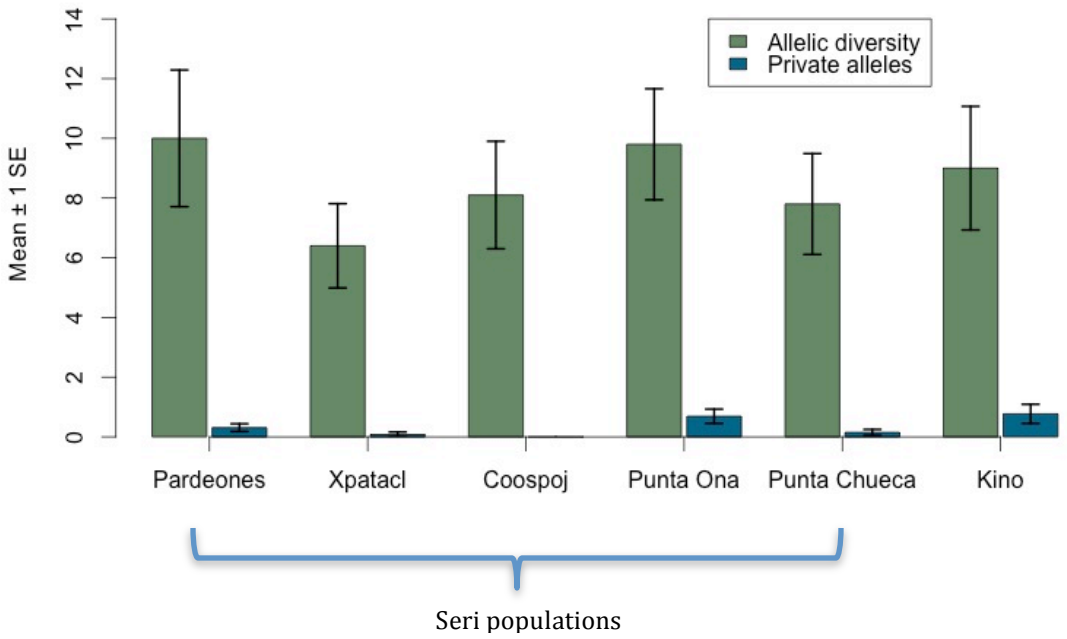



Figure 4. Allelic diversity and private alleles as measured by 13 microsatellite markers in six *A. tuberculosa* populations.

A) At 01

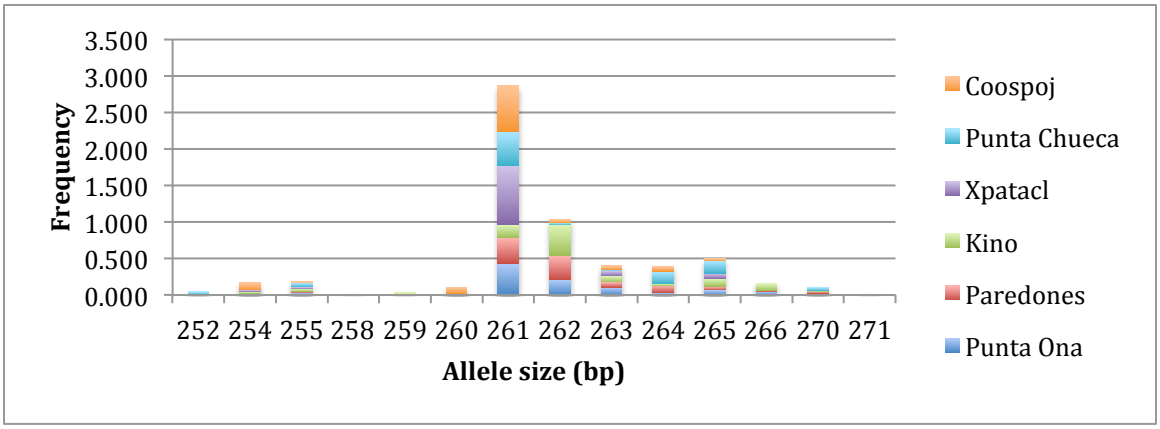
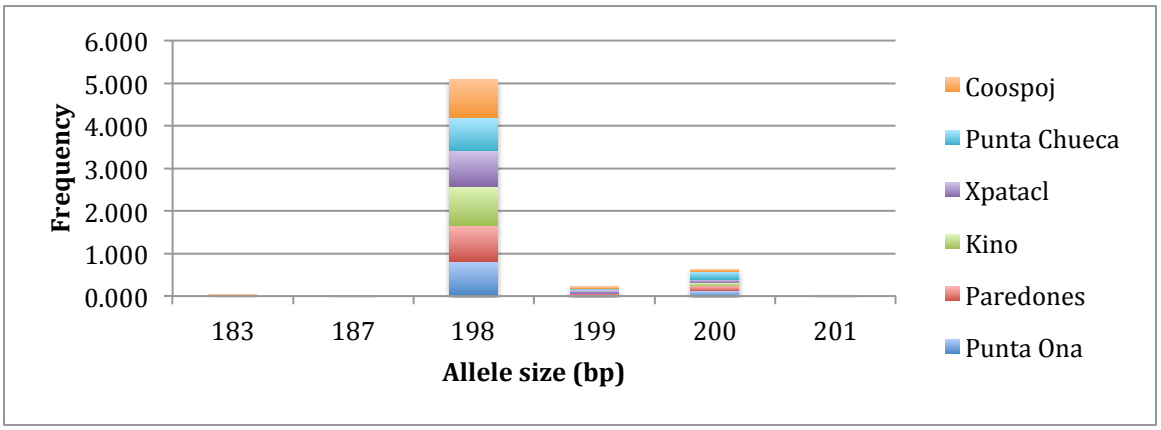
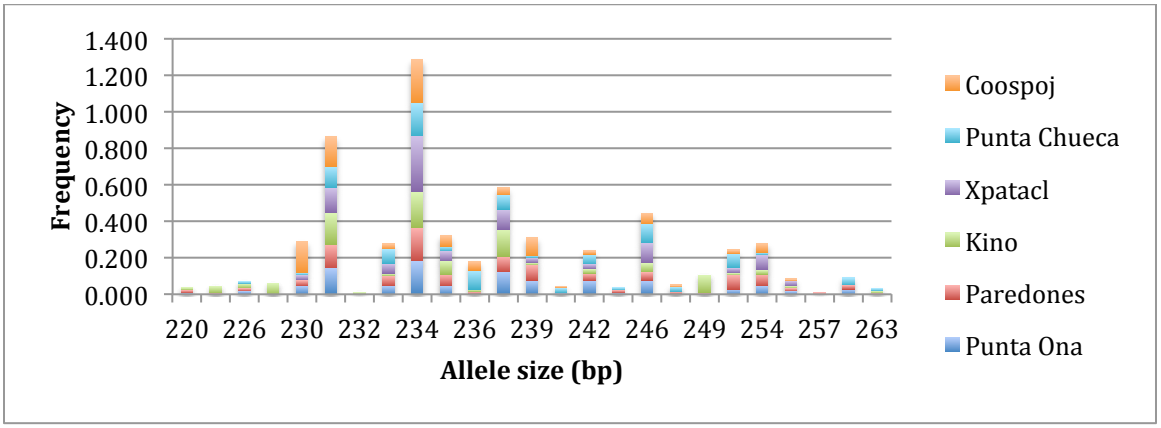



Figure 5. Allelic frequencies across 13 loci in six *A. tuberculosa* populations.

B) At 04




C) At 05




D) At 07

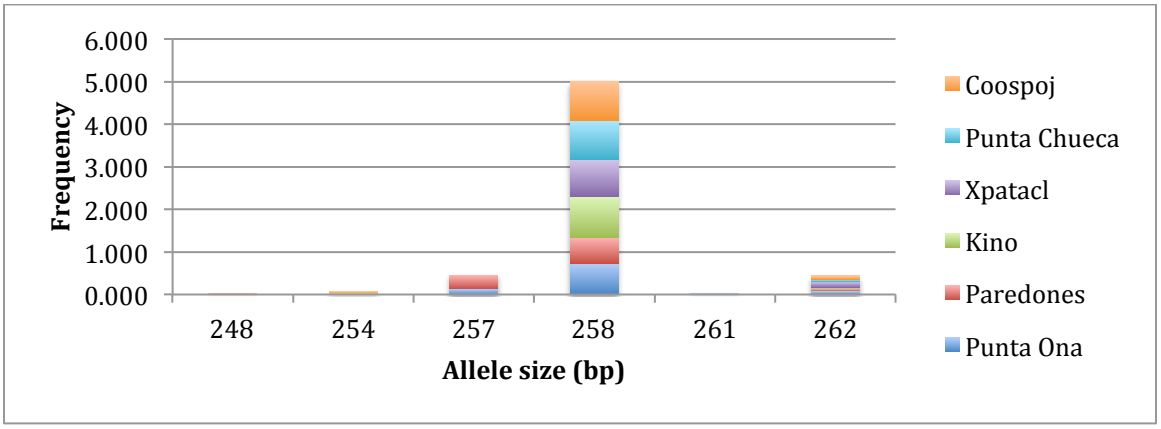
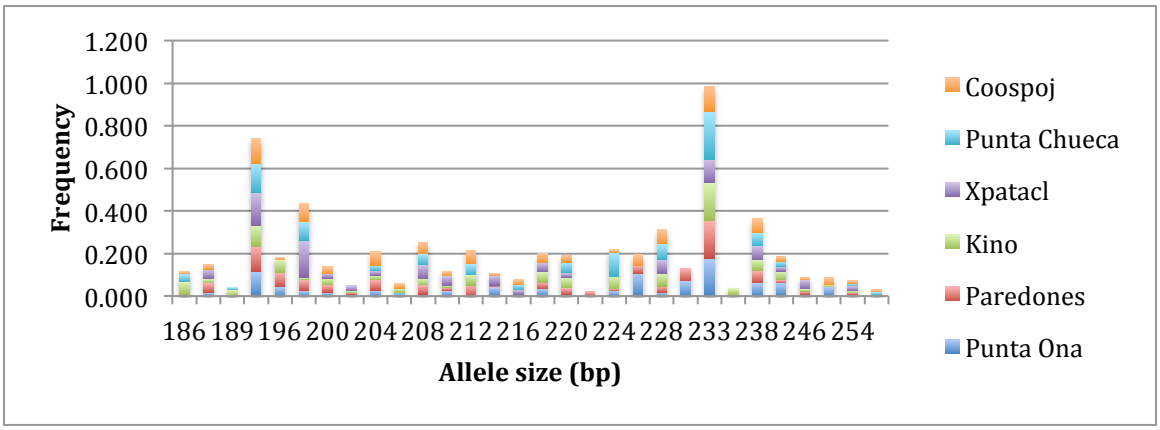
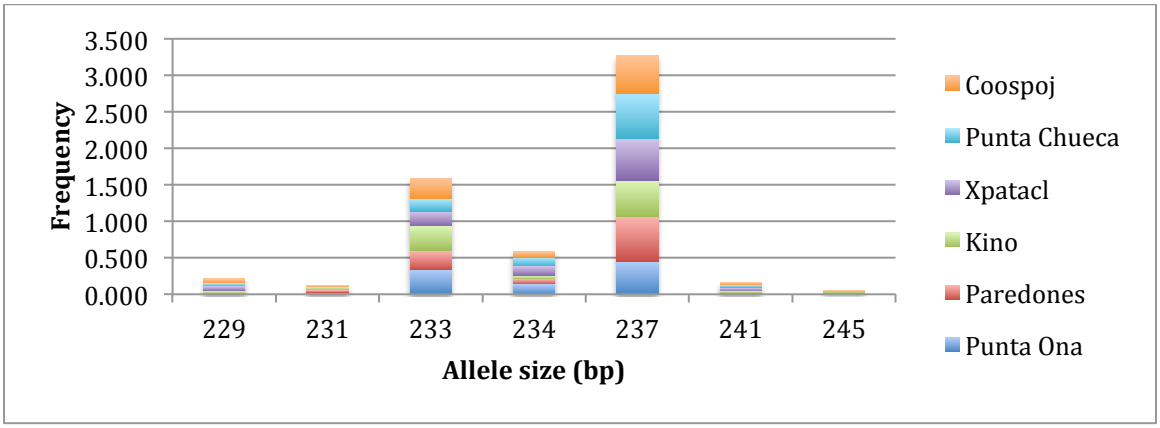



Figure 5 contd. Allelic frequencies across 13 loci in six *A. tuberculosa* populations.

E) At 08




F) At 12




G) At 13

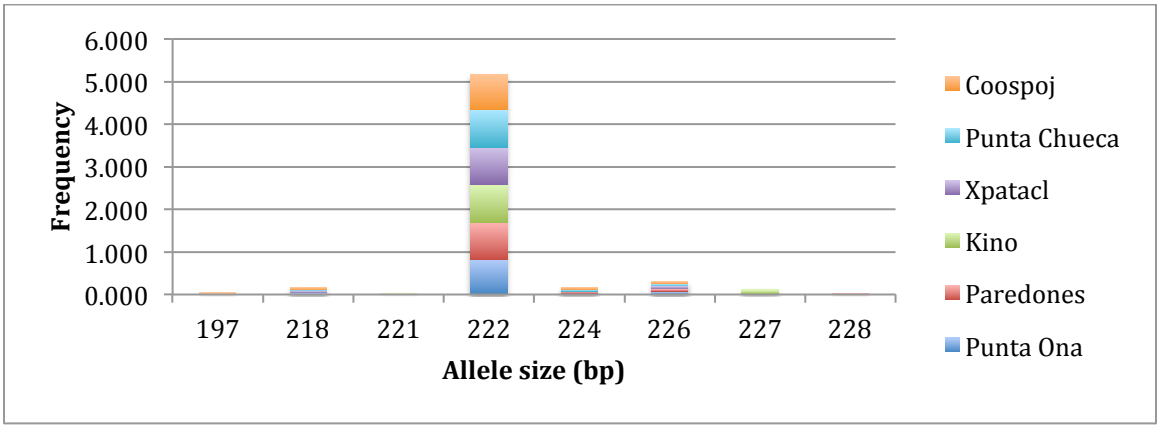
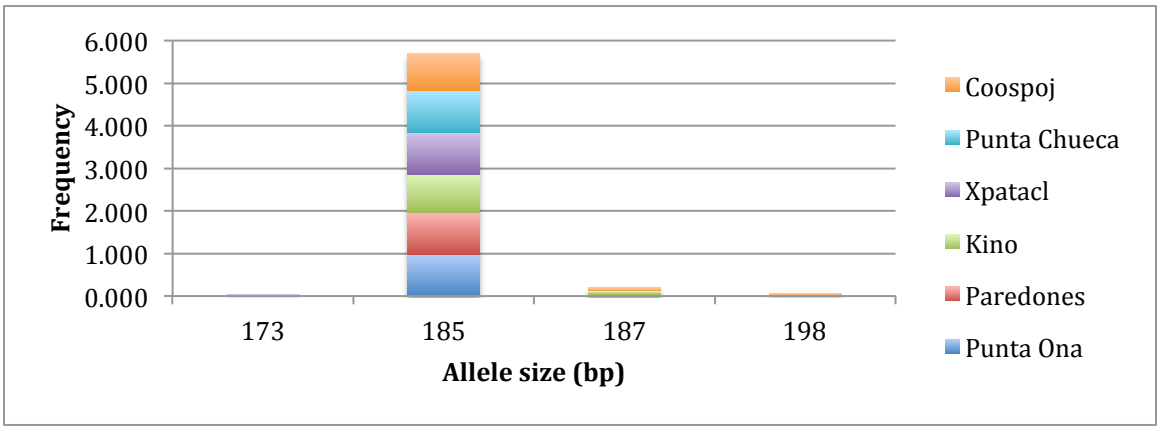
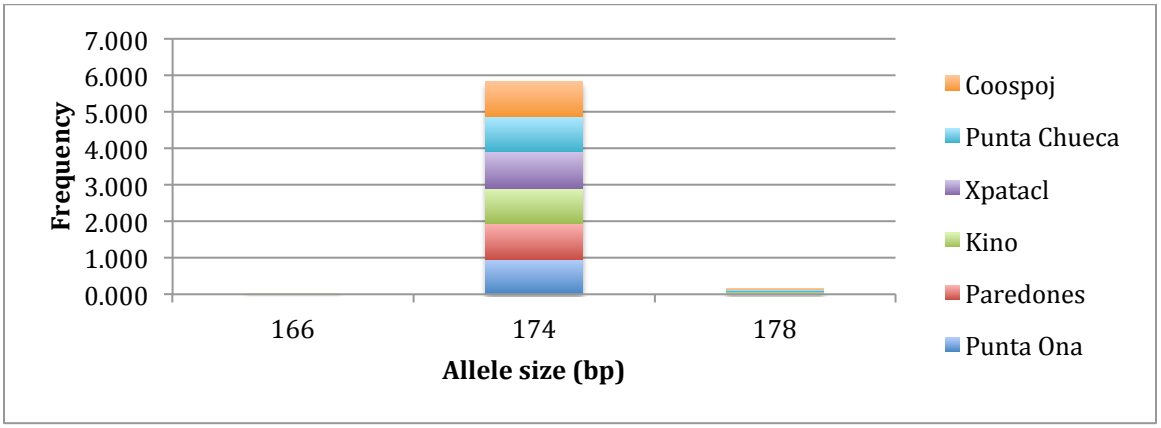



Figure 5 contd. Allelic frequencies across 13 loci in six *A. tuberculosa* populations.

H) At 17




I) At 18




J) At 27

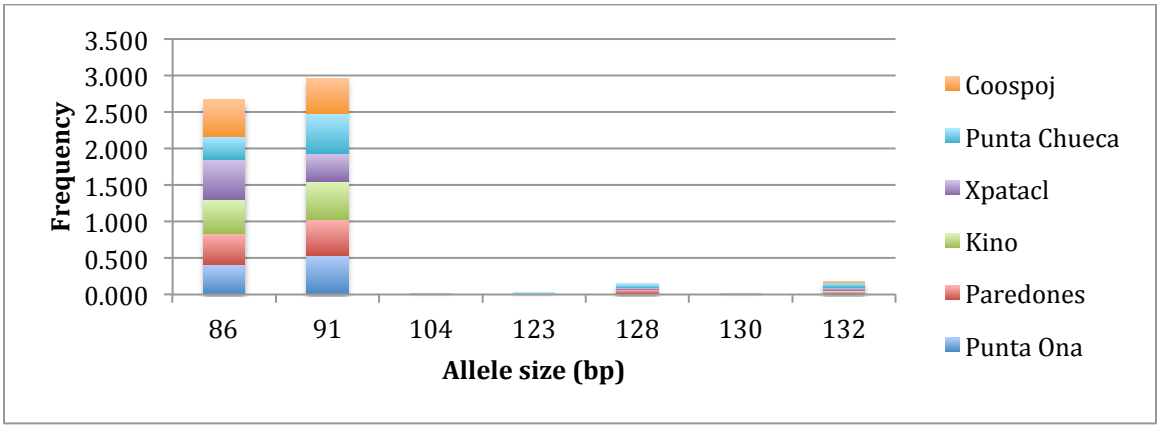
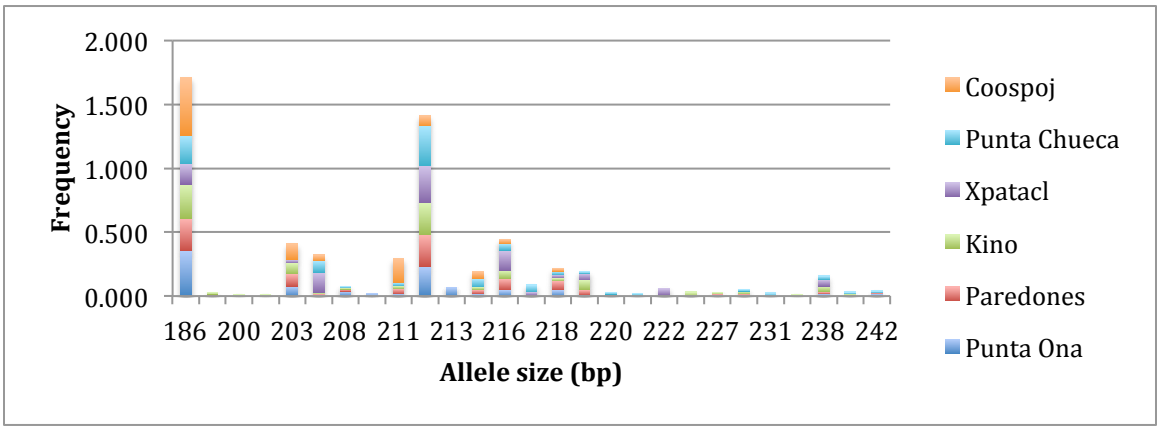
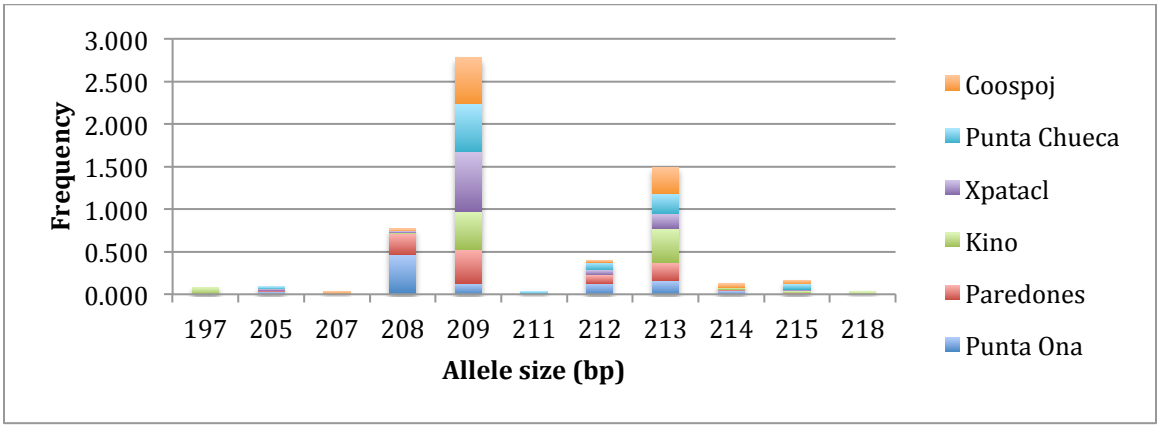



Figure 5 contd. Allelic frequencies across 13 loci in six *A. tuberculosa* populations.

K) At 31




L) At 36




M) At 46

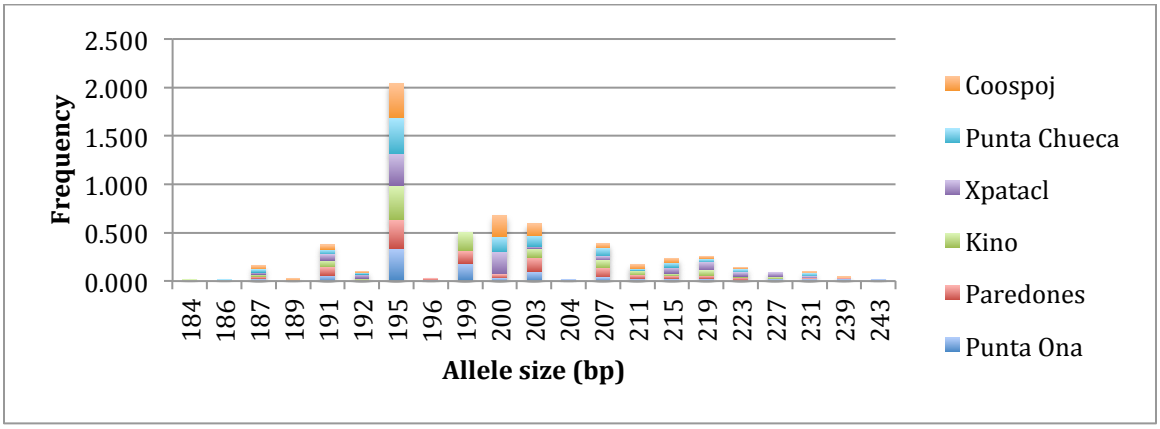



Figure 5 contd. Allelic frequencies across 13 loci in six *A. tuberculosa* populations.

II. Population Differentiation

Pairwise comparison of $F_{ST}$ values (Table 3) revealed significant differences between all population pairs, except for Punta Ona/Paredones and Xpatacl/Punta Chueca, however, a global test of differentiation between all pairs of samples revealed no significant difference among any of the populations.

Analysis of molecular variance (Table 4) indicated that the majority of variation occurs within populations, as opposed to among populations (97% and 3% respectively). Principal component analysis showed no distinct segregations of populations (Figure 6). Additionally, the program STRUCTURE revealed no evidence of population structure or distinct populations (Figure 7).

The major technical issue we faced with this project was the fact that the DNA samples were extracted using two different methods; and, in some cases, the extracted DNA was stored for over a year before being used in PCR. The differing extraction methods resulted in different concentrations of, and we were not able to amplify all 13 microsatellite regions for all individuals. We operated under a threshold of greater than 70% data for each population at each locus, but we may have overlooked some key genetic variation in some of the samples with missing data.

Table 3. Pairwise comparison of population $F_{ST}$ values (*P significant after Bonferroni correction).

| Punta Ona | Paredones | Kino | Xpatacl | Punta Chueca | Coospoj | |
|---|---|---|---|---|---|---|
| 0.00000 | | | | | | Punta Ona |
| 0.00789 | 0.00000 | | | | | Paredones |
| 0.03595* | 0.02664* | 0.00000 | | | | Kino |
| 0.05883* | 0.04187* | 0.05872* | 0.00000 | | | Xpatacl |
| 0.04014* | 0.03353* | 0.02779* | 0.00487 | 0.00000 | | Punta Chueca |
| 0.04250* | 0.03715* | 0.03351* | 0.01574* | 0.01376* | 0.00000 | Coospoj |

Table 4. Analysis of molecular variance (AMOVA) within and among populations.

| Source of variation | d.f. | Sum of squares | Variance components | Percentage of variation |
|---|---|---|---|---|
| Among populations | 5 | 55.771 | 0.08283 | 3.11 |
| Within populations | 628 | 1620.409 | 2.58027 | 96.89 |
| Total | 633 | 1676.180 | 2.66309 | 100 |

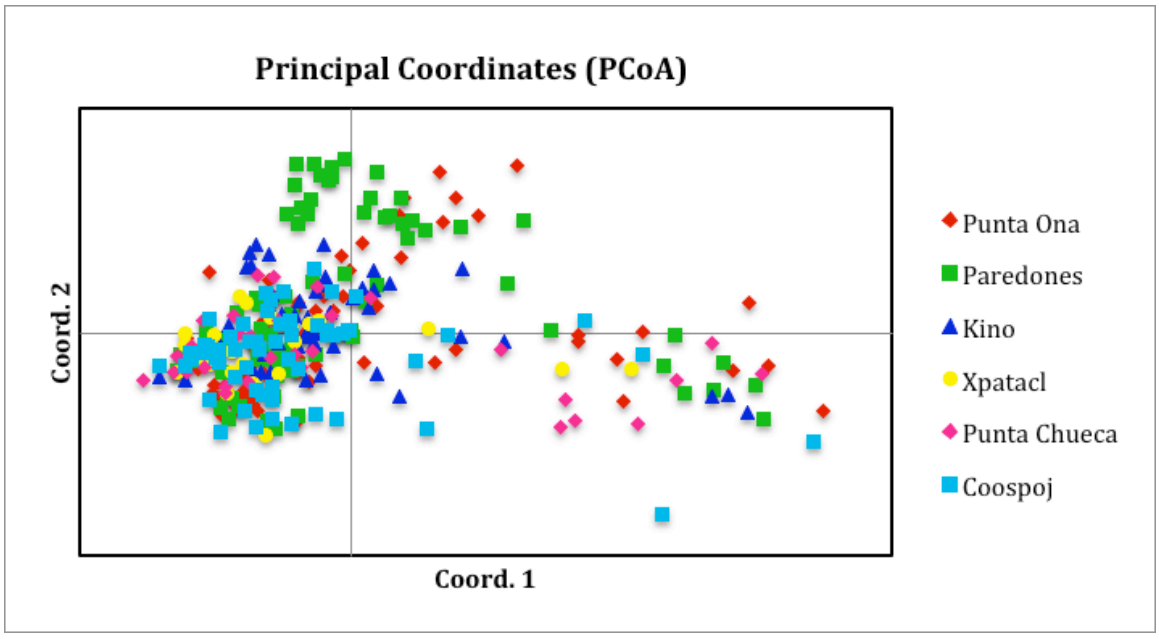
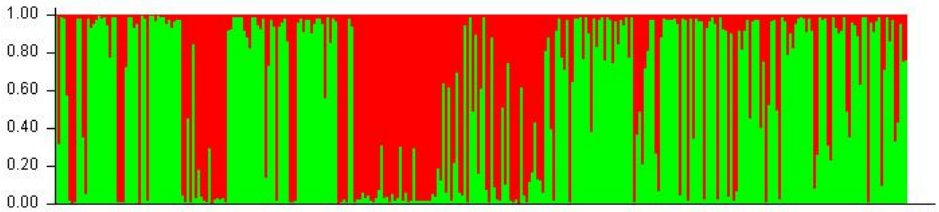
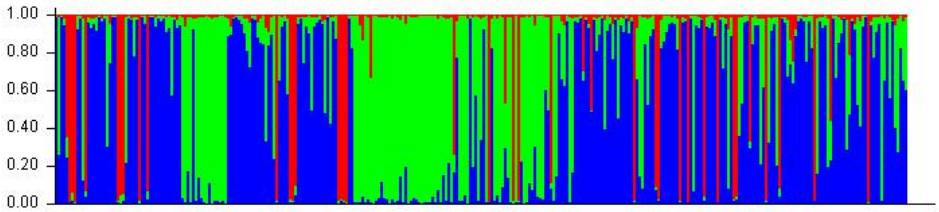



Figure 6. Principal coordinates analysis of microsatellite data pen shells for six sites in the Gulf of California.

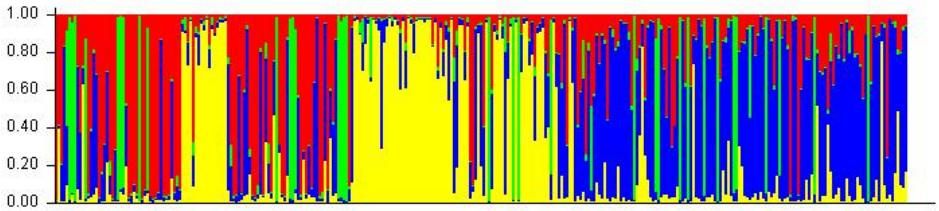
A) K = 2



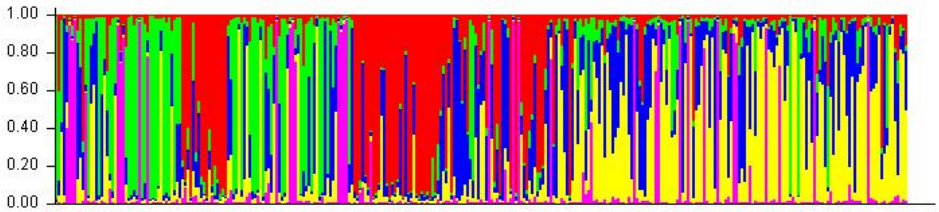

B) K = 3




C) K = 4




D) K = 5




E) K = 6

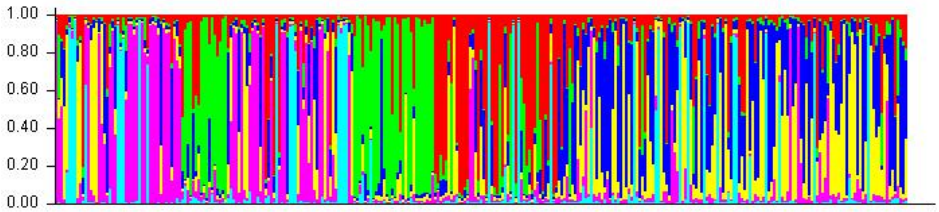



Figure 7. STRUCTURE results showing no segregation of populations using a range of K (discrete populations predicted) from two to six, for pen shell microsatellite data for six locations in the Gulf of California.

## Discussion

The discrepancy in pen shell abundance between two neighboring fisheries that use identical harvesting techniques but which are regulated by different management schemes led us to investigate the levels and possible differences in genetic diversity between these two fisheries. Comparisons across the 13 microsatellite loci tested for six sites in the Gulf of California (Figure 3) showed no significant difference in allelic diversity. Most significantly, no genetic structure differences were observed between the Seri fishery and the Kino fishery assessed using a variety of independent measures (diversity metrics, AMOVA, PCoA, and STRUCTURE analysis). We found very few private alleles present at only a single site and AMOVA attributed 97% of the genetic variation to within-population differences rather than between population differences. Thus, although we have shown that there is gene flow between these two fisheries, potential larval influx from the Infiernillo Channel into Kino Bay is not enough to maintain harvesting levels under the current management practices.

The lack of genetic differentiation between the sites implies that there is gene flow between the Seri fishery and the Kino fishery; and because of pen shell reproductive biology, it can be assumed that this flow is by means of larval transport. The fact that nearly all of our loci deviated from the expected Hardy-Weinberg equilibrium indicates non-neutral genetic variation in our samples. The program Microchecker suggests that this deviation is due to the presence of null alleles, meaning that there may be a mutation in the region flanking the microsatellite regions preventing the amplification of some alleles. So, while we

found no significant difference in genetic diversity among any of the six populations, the values we report for total allelic diversity (A) may be lower than is actually present in these populations. The highest number of private alleles was found in the Kino population, which could be a product of stochasticity, but could also suggest directionality of larval transport, which would be an interesting area of future research.

We have shown that there is genetic exchange between the Seri and the Kino fisheries, and, in fact, these two fisheries' management practices are acting upon a single, contiguous stock of pen shell. Despite the fact that these two fisheries are genetically connected, the Kino fishery reports a decline in abundance of pen shell, while the Seri fishery has remained stable, which supports the idea that management practices may be an important factor in the discrepancy in abundance (Basurto et al. 2012). Returning to the idea of marine protected area providing a benefit to nearby fisheries in terms of a genetic or larval influx, here we found that although larval transport connects the sites genetically, it is not sufficient to sustain the Kino fishery under its current management practices.

These results are in line with the findings of Takashina and Mougi (2014) who mathematically modeled the effects of marine protected areas on overfished stocks and found that MPAs had little to no effect on the equilibrium population size of sessile species. They also highlight the importance of management practices in the efficacy of an MPA, suggesting that the establishment of an MPA may transfer and concentrate fishing effort into areas outside of the protected area, which, in their model, reduced MPA efficacy. In our study, the protected area has been in place

for more than 30 years; however the number of fishermen utilizing the Kino fishery is double that of the Seri. Thus, this high concentration of fishers in Kino may cancel out any positive effect the adjacent Seri fishery provides.

West et al. (2009) also used a population model to investigate the effects of marine reserves on fisheries. They found that the efficacy of MPAs is negatively correlated with level of migration, leading me to suggest that an area of interesting future research would be to model currents and tidal patterns in the Gulf of California in order to gain a better understanding of the dispersal (migration) range of the pen shell during its planktonic larval phase. I also would recommend expanding the geographic range of this population genetics study to include more fishing areas, both to the north of Tiburón Island and to the south of Kino Bay in order to investigate gene flow at a larger scale. Because this study provides baseline data for genetic diversity in these two fisheries, these data can also be used to monitor levels of genetic diversity over time.

One of the major limitations of this study is the fact that 5 of our 6 sample sites are in the Seri fishery, while only one is in the Kino fishery. This is because the Seri have anecdotally reported more variation in pen shell morphology and we wanted to focus our efforts on capturing genetic diversity inside the Infiernillo Channel. Again, expanding the geographic range of this study to include more Kino sites, as well as sites to the north and the south of these two fisheries, will provide a more comprehensive understanding of the levels of genetic diversity in the total population.

We have shown that there is, in fact, no significant genetic structure or difference in genetic diversity between the Seri and the Kino pen shell fisheries, indicating that there is positive gene flow by means of larval transport. Considering the Seri fishery to be a community-based MPA, we have shown that any influx of larvae into Kino Bay from the Infiernillo Channel is not sufficient to sustain the population of pen shells in Kino Bay under the current harvesting regime. Therefore, it will be necessary to modify the management practices in the Kino Bay fishery if this population is to persist.

**Acknowledgements**

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